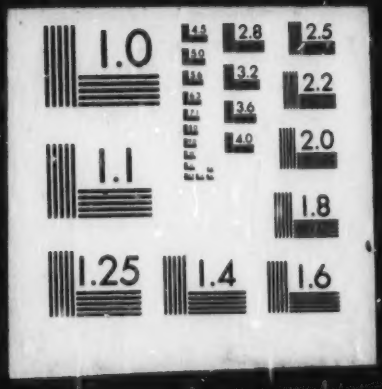
1 OF 1
PB
262 233



U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-262 233

Biological Electrodes Specific for Phosphate and Nitrite Ions

New Orleans Univ, La

Prepared for

Environmental Research Lab, Athens, Ga

Oct 76

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/4-76-034		

4. TITLE AND SUBTITLE
Biological Electrodes Specific for Phosphate and Nitrite Ions

5. REPORT DATE
October 1976 (Issuing date)

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
G. G. Guilbault

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of New Orleans
Lake Front
New Orleans, Louisiana 70122

10. PROGRAM ELEMENT NO.
1BA027

11. CONTRACT/GRANT NO.
R 800359

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Athens, Georgia 30601

13. TYPE OF REPORT AND PERIOD COVERED
Final

14. SPONSORING AGENCY CODE
EPA-ORD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Biological electrodes have been developed for the selective analysis of phosphate and nitrate ions at micro-levels. A phosphate electrode was constructed by using two enzymes, alkaline phosphatase and glucose oxidase which were immobilized and mounted on the surface of the Platinum electrode. The competitive inhibition of phosphate ion on alkaline phosphatase slows down the hydrolysis of glucose-6-phosphate to glucose which is subsequently used by glucose oxidase as a substrate in the presence of molecular oxygen. The phosphate ion concentration in the reaction mixture was found to be proportional to the oxygen consumption.

The assay of nitrite was accomplished with the use of nitrite reductase which catalyzes the reduction of nitrite into ammonia. Nitrite in the range of 10^{-4}M-10^{-2}M could be accurately determined with either soluble or immobilized enzyme using an air-gap electrode as sensor.

Preparations of benzidine phosphate and hexammino cobalt nitrate in silicone rubber for liquid ion exchange and triphenyltin electrode have been attempted and results obtained were unsatisfactory due to their poor selectivity.

This report was submitted in fulfillment of R 800359-02 by University of New Orleans under the sponsorship of the Environmental Protection Agency. Work was completed as date of 12-31-75.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
*chemical analysis *electrodes *ion-exchangers alkaline phosphatases enzymes nitrites perchlorates	*ion-selective phosphate glucose oxidase biological electrode nitrite reductase	05A

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	UNCLASSIFIED	14
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE 3.50-3.00

*USGPO: 1976 -- 757-056/5422 Region 5-11

PB 262 233

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/4-76-034
October 1976

BIOLOGICAL ELECTRODES SPECIFIC FOR PHOSPHATE AND NITRITE IONS

by

George G. Guilbault
University of New Orleans
New Orleans, Louisiana 70122

Number R-800359

Project Officer

Thomas B. Hoover
Analytical Chemistry Branch
Environmental Research Laboratory
Athens, Georgia 30601

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30601

DISCLAIMER

This report has been reviewed by the Athens Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

Biological electrodes have been developed for the selective analysis of phosphate and nitrite ions at micro-levels. A phosphate electrode was constructed by using two enzymes, alkaline phosphatase and glucose oxidase, which were immobilized and mounted on the surface of the platinum electrode. The competitive inhibition of phosphate ion on alkaline phosphatase slows down the hydrolysis of glucose-6-phosphate to glucose, which is subsequently used by glucose oxidase as a substrate in the presence of molecular oxygen. The phosphate ion concentration in the reaction mixture was found to be proportional to the oxygen consumption.

The assay of nitrite was accomplished with the use of nitrite reductase which catalyzes the reduction of nitrite into ammonia. Nitrite in the range of 10^{-4}M-10^{-2}M could be accurately determined with either soluble or immobilized enzyme using an air-gap electrode as sensor.

Preparations of benzidine phosphate and hexammino cobalt nitrate in silicone rubber for liquid ion exchange and triphenyltin electrode have been attempted and results obtained were unsatisfactory due to their poor selectivity.

This report was submitted in fulfillment of R 800359-02 by University of New Orleans under the sponsorship of the Environmental Protection Agency. Work was completed as date of 12-31-75.

ACKNOWLEDGMENTS

Three post doctoral associates, Dr. M. Nanjo, Dr. T. J. Rohm and Dr. S. S. Kuan, were employed on the project. One graduate student, Mr. C. H. Kiang, was employed, doing basic research associated with his Ph.D. degree to be received next year.

This support from EPA is gratefully acknowledged, as is the kind assistance and advice of Dr. Thomas Hoover, of the Environmental Research Laboratory in Athens, Georgia.

CONTENTS

Sections		Page
I.	Conclusions	1
II.	Recommendations	2
III.	Introduction	3
IV.	Results and Discussion	4
V.	References	7

SECTION I

CONCLUSIONS

Attempts to employ an insoluble salt or ligand-association complex for the selective assay of phosphate ion have been made. Among the salts tested, good sensitivity was achieved with the phosphonium and triphenyltin salts, but both lacked sufficient selectivity, therefore, the use of these substances in electrodes for standard routine assay of phosphate is not recommended. However, the use of these electrodes as an aid to double check the phosphate ion concentrations at micro-levels is recommended. On the other hand, the use of extremely selective phosphate and nitrite enzyme electrodes for the detection of PO_4^{3-} and NO_2^- is highly suggested for routine analysis due to their good sensitivity and selectivity. These electrodes are highly selective for these ions.

SECTION II

RECOMMENDATIONS

It is recommended that further work be done on these electrodes, to insure their widespread use in environmental sampling for nitrite and phosphate. Prototypes have been developed, but additional work must be done to evaluate these electrodes, and perfect them for experimental use in everyday situations.

SECTION III

INTRODUCTION

The objective of this research project was to develop selective-ion electrodes for phosphate and nitrite ions that can be used for the continuous monitoring of these ions at microlevels. Because of the great sensitivity of enzyme systems (as little as 10^{-12}g of some substances is determinable) such systems have proven useful for the assay of various substances. Enzyme systems can be used to build in selective electrodes for substances for which selective conventional electrodes cannot be made. Phosphate and nitrate ions are pollutants commonly present in water, being discharged from detergents, fertilizers and other sources. We took two approaches to this problem. The first was the development of an electrode that employed an insoluble salt as the sensing element. The second approach involved the use of enzyme electrodes in which the enzyme catalyzed a reaction involving phosphate or nitrite ion and the reaction was followed electrochemically.

SECTION IV

RESULTS AND DISCUSSION

The preparation of a solid-state electrode for orthophosphate has been reported (1). This electrode was prepared by mixing a glutaraldehyde-thiourea polymer with silver ion and dibasic phosphate to obtain an insoluble complex (PCPX-1). This material was mixed with silver sulfide and pressed into a pellet which is then used as the sensing membrane of an electrode. This electrode responds to dibasic phosphate concentration over the range 10^{-1} to 10^{-4} M. Sulfate ion interfers with the performance of the electrode and the device loses sensitivity after 48 hours. To improve the stability and selectivity of the solid-state electrode, we investigated the use of derivatives of thiourea (i.e., thiocarbanilide); 1,3-didecyl-2-thiourea; 2,2'-diethylthiocarbanalide; 1,3-diethyl-2-thiourea and 1,1,3,3-tetramethyl-2-thiourea) for the preparation of silver complexes similar to PCPX-1. All of these compounds yielded silver sulfide and various organic products when treated with silver nitrate. Electrodes prepared from these products in the same manner as the original solid-state electrode have essentially the same properties as the PCPX-1 electrode. Since no improvement was made, this phase of investigation was not pursued.

The use of ligand-association complexes of 2,2'-bipyridine; 1,10-phenanthroline and related compounds with transition metals as exchange materials for the preparation of liquid ion-exchange electrodes has been fully investigated. Liquid-type electrodes prepared from these complexes were sensitive to ClO_4^-, I^-, Br^-, NO_3^- and Cl^-, generally in the order cited. A limited response to orthophosphate was achieved by converting the trisbathophenanthroline iron(II) complex to the orthophosphate form and using the resulting product to construct a liquid ion-exchange electrode. During the study of methods for the preparation of liquid ion-exchange electrodes, we found that incorporation of the exchange solution in poly(vinyl chloride), PVC, improved the performance of the electrode. We reported these findings in Reference 2 and gave a summary of the results of studies of solid-state and ligand-association complex electrodes at the ACS National Meeting in Dallas, April, 1973.

During the period covered by this grant, the investigator prepared a report for IUPAC (3), and reviewed the field of ion-selective electrodes (4).

We studied the possibilities of using the reaction of benzidine and various complex salts of cobalt(III) as membrane materials.

These compounds are selective for phosphates, but were found to lack the mechanical and electrical properties necessary for the preparation of ion-selective electrode.

The next area investigated involved the use of quarternary ammonium salt as exchange materials for the preparation of a phosphate selective electrode (5). The quaternary ammonium salt, General Mills Aliquat 3365 (methyltricapryl-ammonium chloride), was used to prepare a divalent phosphate electrode (6) and electrodes for a variety of anions have been reported (7) using the same salt after convercion of the anion to that appropriate for the type of electrode constructed. We prepared similar electrodes which showed linear response to orthophosphate over the range 10^{-1} to 10^{-4} M at pH 4.3. These electrodes employed quaternary ammonium phosphate salts as exchange materials. The stability of such electrodes is excellent, but the selectivity is poor with selectivity coefficients greater that unity for common ions (Cl^-, OAc^-, Br^-). However, the nature of the organic groups which are attached to the central nitrogen atom greatly influences the selectivity of such electrodes and we were able to construct electrodes with varying selectivities by changing the type of quaternary ammonium salt used. The selectivity is closely related to extraction coefficients as has been recently reported (8, 9).

In conclusion, precipitate-type electrodes (benzidine sulfate or phosphate and hexammino-cobalt(III) nitrate in silicone rubber), and liquid ion-exchanger electrodes based on quaternary, ammonium and phosphonium and triphenyltin salts were tested for the assay of phosphate ions. Good sensitivity was achieved with the phosphonium and triphenyltin salts, but both lacked sufficient selectivity for routing assays (5).

An enzyme electrode which senses oxygen consumption for the assay of phosphate ion (10^{-3}-10^{-4} M) was constructed by using two enzymes together:

$$\text{glucose-6-phosphate} \xrightarrow{\text{alkaline phosphatase}} \text{glucose} + \text{phosphate}$$

$$\text{glucose} \xrightarrow[O_2]{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

The competitive inhibition by phosphate ion added caused a smaller and slower oxygen consumption which could be detected by a platinum disc electrode at -0.6 V vs. SCE amperometrically. This dual enzyme electrode was also found useful for the assay of

oxyacids other than phosphate, such as arsenic, tungstate, molybdate and borate (10). The electrode is completely specific for phosphate under controlled conditions, and is the best electrode thus far developed for phosphate ion.

The enzyme, nitrite reductase, was extracted and purified from spinach leaves; the freeze-dried preparation is completely stable for at least 4 months if kept in a freezer. The enzyme catalyzes the reduction of nitrite to ammonia in the presence of reduced methyl viologen as electron donor. An assay of nitrite can be based on the measurement of the ammonia formation, with an air-gap electrode as sensor. Nitrite in the 10^{-4} M-5 x 10^{-2} M range can be accurately determined with either soluble or immobilized enzyme, but the latter is stable for at least three weeks, is less susceptible to interferences during assay, and can be used repeatedly for about a hundred runs. These advantages make the method very simple, valuable and economical for the routine analysis of nitrite ion (11).

SECTION V

REFERENCES

1. Shu, F. R. and G. G. Guilbault, An Ion-Selective Electrode for Dibasic Phosphate Ion. Anal. Lett. 5(8), 559-565 (1972).

2. Rhom, T. J. and G. G. Guilbault, New Methods for the Preparation of Perchlorate Ion-Selective Electrodes. Anal. Chem. 46, 590-592 (1974).

3. Guilbault, G. G., Recommendations for Nomenclature of Ion-Selective Electrodes. IUPAC Report, 1975.

4. Guilbault, G. G. and T. J. Rhom, Ion-Selective Electrodes and Enzyme Electrodes in Environmental and Clinical Studies. Intern. J. Environ. Anal. Chem. 4, 51-64 (1975).

5. Nanjo, M., Timothy J. Rhom and G. G. Guilbault, An Investigation of Polyphenyl-Onium Bases and Other Materials for Phosphate Ion-Selective Electrodes. Anal. Chim. Acta. 77, 19-27 (1975).

6. Nagelberg, I., L. I. Braddock, and G. J. Barbero. Divalent Phosphate Electrode. Science 166 (3911), 1403-1404, December 1969.

7. Coetzee, C. J. and H. Freiser. Liquid-Liquid Membrane Electrodes Based on Ion Association Extraction Systems. Anal. Chem. 41(8), 1128-1130, July 1969.

8. Reinhoudder, R. E. and F. A. Schultz. Anion Selectivity Studies on Liquid Membrane Electrodes. Anal. Chim. Acta (Netherlands) 65, 425-435 (1973).

9. Back, S. and J. Sandblom. Role of Solvent Extraction Parameters in Governing the Potential Selectivity of Liquid Membrane Electrodes. Anal. Chem. 45(9), 1680-1684, August 1973.

10. Guilbault, G. G. and M. Nanjo, A Phosphate-Selective Electrode Based on Immobilized Alkaline Phosphatase and Glucose Oxidase. Anal. Chim. Acta 78, 69-80 (1975).

11. Kiang, Chih-Hen, S. S. Kuan, and G. G. Guilbault, A Novel Enzyme Electrode Method for the Determination of Nitrite Based on Nitrite Reductase. Anal. Chim. Acta 80, 209-214 (1975).